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Brelsford, Craig

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Do UV-A radiation and blue light during growth prime leaves to cope with acute high-light in photoreceptor mutants of *Arabidopsis thaliana*?

Craig C. Brelsford[1], Luis O. Morales[1], Jakub Nezval[2], Titta K. Kotilainen[1], Saara M. Hartikainen[1], Pedro J. Aphalo[1] and T. Matthew Robson[1]✉

Affiliations

[1] Organismal and Evolutionary Biology Research Programme, Viikki Plant Science Centre (ViPS), Faculty of Biological and Environmental Sciences, 00014, University of Helsinki, Finland.

[2] Faculty of Science, University of Ostrava, 30. dubna 22, 701 03 Ostrava, Czech Republic.

Correspondence email: matthew.robson@helsinki.fi

Abstract (244/250 Words)

We studied how plants acclimated to growing conditions that included combinations of blue light and ultraviolet-A (UV-A) radiation, and whether their growing environment affected their photosynthetic capacity during and after a brief period of acute high light (as might happen during an under-canopy sunfleck). *Arabidopsis thaliana* wild-type Landsberg *erecta* (WT) were compared with mutants lacking functional blue-light-and-UV photoreceptors: phototropin 1 (phot1); cryptochromes (cry1 and cry2) and UV RESISTANT LOCUS 8 (uvr8). This was achieved using LED lamps in a controlled environment to create treatments with or without blue light, in a split-plot design with or without UV-A radiation. We compared the accumulation of phenolic compounds under growth conditions and after exposure to 30 minutes of high light at the end of the experiment (46 days), and likewise measured the operational efficiency of photosystem II (φPSII a proxy for photosynthetic performance) and dark-adapted maximum quantum yield (F_v/F_m to assess PSII damage). Our results indicate that cryptochromes are the main photoreceptors regulating phenolic-compound accumulation in response to blue light and UV-A radiation, and a lack of functional cryptochromes impairs photosynthetic performance under high light. Our findings also reveal a role for UVR8 in accumulating flavonoids in response to a low UV-A dose. Interestingly, phototropin 1 partially-mediated constitutive accumulation of phenolic compounds in the absence of blue light. Low irradiance blue light and UV-A did not facilitate higher φPSII and F_v/F_m to our acute high light treatment, however CRYs played an important role in ameliorating high-light stress.

Abbreviations

CHS, chalcone synthase ; *cry1 cry2*, cryptochrome 1,2; ϕPSII, Operating efficiency of photosystem II; F_v/F_m , Maximum quantum efficiency of PSII; BL, Blue light – 420-490nm; B:G – ratio of blue:green light defined as 420-490:500-570nm, HPLC-DAD, High performance liquid chromatography coupled with a diode array detector; PAR, Photosynthetically active radiation (400nm-700nm); *phot1*, phototropin 1 mutant; PSII, Photosystem II; R:FR, Red : Far-red light ratio defined as 655-665:725-735m; SAS, Shade avoidance syndrome; UV, Ultraviolet radiation – 100-400nm; UV-A, Ultraviolet A radiation – 315-400nm; UV-B, Ultraviolet B radiation – 280-315nm; *uvr8-2*, UV Resistance locus 8-2 mutant; WT, Landsberg *erecta* wild type; VPD, Vapour pressure deficit.

Introduction

Plants experience a dynamic and heterogeneous light environment, where spectral composition and irradiance can change depending upon the time of day, and of year, as well as the surrounding vegetation (Constabel and Lieffers 1996, Montgomery and Chazdon 2001). To maintain a positive carbon balance, plants beneath canopies must respond to changes in solar radiation throughout the growing season (Augspurger 2003, Lopez et al. 2008, Dion et al. 2016). As well as reduced irradiance during spring due to canopy closure, differential attenuation of the blue (Casal 2013a; who define blue as 400 − 500 nm) and UV-A regions affect spectral composition in the understorey (Grant et al. 2005). Spring bud burst of deciduous species in temperate and boreal forests results in a reduction in total under-canopy irradiance (Richardson and O'Keefe 2009) and a change in its spectral composition; reductions in the R:FR and blue:green ratios, and an increase in the UV:PAR ratio reaching the understorey (Flint and Caldwell 1998, Leuchner et al. 2011, Urban et al. 2012, Dengel et al. 2015). In response to increases in irradiance, plants produce phenolic compounds such as anthocyanins and phenolic acids (Agati et al. 2012). For instance, flavonoid glycosides can serve as sun-screens accumulating in the vacuoles, and bound to cell walls, of leaf epidermal cells protecting the mesophyll from photo-bleaching and photosynthetic damage (Jansen et al. 1998, Barnes et al. 2008). Flavonoids absorb UV radiation, whilst also functioning as antioxidants preventing damage from reactive oxygen species (Agati and Tattini, 2010). While the low UV irradiance under most canopies is unlikely to be harmful to plants, it might be exploited as a cue to optimise growth and defence to suit the environment (Mazza and Ballaré 2015).

Plants monitor their light environment through perception of specific regions of the spectrum, and coordinate a response that adjusts their growth strategy to suit the conditions (Casal 2013a, Casal 2013b). Different photoreceptors react to specific regions of the spectrum but often interact permitting the plant to be sensitive to complex changes in spectral irradiance (Heijde and Ulm, 2012). Plant photoreceptors cry1 and cry2, and phot1 and phot2, primarily absorb photons in the blue and UV-A regions of the spectrum (Briggs and Huala 1999, Banerjee and Batschauer 2005). Of these, phot1 and phot2, are involved in stomatal opening, tropism and hypocotyl elongation responses (Casal 2000). Specifically, phot1 promotes cryptochrome-mediated accumulation of anthocyanins in response to blue light (Kang et al. 2008), and phot2 regulates the chloroplast-avoidance response to high light in angiosperms (Briggs and Christie 2002, Litthauer et al. 2015). To date, neither phot1 nor phot2 has been reported to regulate the pathways responsible for the accumulation of flavonoids. Photoreceptors, cry1 and cry2, alongside phyA and phyB detect the light cues which entrain the circadian clock (Somers et al. 1998). Hypocotyl elongation, seedling development, as well as the accumulation of flavonoids and anthocyanins are also regulated by cry1 and cry2 (Kubasek et al. 1992; Casal 2000, Shalitin et al. 2002). There is also evidence that cry1 interacts with phyB, which detects changes in R:FR ratio in the presence of neighbouring plants, eliciting

the SAS response (Ballaré et al. 1987, , Ballaré et al. 1990 Keller et al. 2011). Perception of UV-B through UVR8 attenuates the SAS by supressing stem elongation, in an antagonistic response to that triggered by phyB under FR (Mazza and Ballaré 2015). UVR8 also promotes the pathways for accumulation of flavonoids in response to UV-B radiation under controlled conditions (Kliebenstein et al. 2002) and at the solar UV-B irradiance occurring outdoors during July in Finland (Morales et al. 2013).

UV-B radiation has consistently been found to up-regulate the accumulation of flavonoids in plants, both in controlled experiments using UV-B lamps (Ballaré et al. 1995, Demkura & Ballaré 2012), and in experiments conducted outdoors under solar UV-B radiation (Flint et al. 1985, Krizek et al. 1998, Morales et al. 2013). Although flavonoid accumulation in *A. thaliana* in response to UV-B has been well studied, less is known about the influence of the blue and UV-A regions of the spectrum on this response (Christie and Jenkins 1996, Fuglevand et al. 1996, Morales et al. 2013). UV-A radiation constitutes a greater proportion of the received solar irradiance than UV-B radiation, but is not attenuated by the ozone layer so has not been subject to such intensive research. There is evidence that some flavonoid groups are regulated mainly by UV-A and others predominately by UV-B (Kotilainen et al. 2008, Siipola et al. 2015, Verdaguer et al. 2017). For instance, in an outdoor experiment filtering out solar UV-A and UV-B, Kotilainen et al. (2008) found specific UV-A and UV-B effects on the accumulation of flavonols, flavones and cinnamic acids in *Betula pendula* and *Alnus incana*. Similarly, in an outdoor experiment filtering out different wavelengths of solar radiation, blue light had stronger effects than UV-A radiation on the accumulation of epidermal flavonol content in pea (*Pisum sativum*) (Siipola et al. 2015). Nevertheless, both UV-A radiation and blue light can elicit flavonoid-accumulation to varying degrees. Morales et al. (2013) suggested that the UV-A/blue light signalling pathway, most likely mediated by crys, maybe interacting with UVR8 to modulate UV-A responses such as flavonoid accumulation. However, there has been little research comparing the responses of plants to UV-A radiation with and without blue light, to examine whether these interactions between photoreceptor responses involve redundancy or fine-tuning between blue and UV-A radiation to regulate flavonoid accumulation.

Although PAR defines the spectral region of radiation useful for photosynthesis (McCree 1981), beyond this region UV-A radiation has also been reported to increase the rate of photosynthesis in over 100 plant species across a variety of life forms (Turnbull et al. 2013), including *A. thaliana* (Bilger et al. 2007). Chlorophylls (Chl) *a* and *b* can absorb radiation within the UV-A region (Lang and Lichtenthaler 1991, McCree 1981), supporting the suggestion that this region also has the potential to drive photosynthesis. However, epidermal screening by UV-absorbing compounds such as flavonoids often drastically reduces the amount of UV-A radiation reaching Chl *a* and Chl *b* in the mesophyll (Bilger et al. 1997). This means that UV-A radiation may only be exploited as an alternative energy source for photosynthesis in low light environments when plants have low concentrations of epidermal UV-

absorbing compounds (Štroch et al. 2015).

UV-A radiation can have deleterious effects on photosynthesis (Jansen et al. 1998, Booij-James et al. 2000). Both UV-A and UV-B radiation can cause damage to the PSII protein complex and reduce quantum efficiency (Jansen et al. 1998); in doing so they increase photoinhibition of PSII more, per photon, than excess PAR (Turcsányi and Vass 2000). However this effect is somewhat counterbalanced by the fact that, UV-A radiation, as well as blue light, also enhance DNA-repair (Jansen et al. 1998, Booij-James et al. 2000). Entrainment of the circadian rhythm of the photosynthetic capacity of PSII under low or dynamic light conditions requires phot1 and phot2, acting through mechanisms that are yet to be fully elucidated (Litthauer et al. 2015). The role of phot2 in the chloroplast avoidance response, permits avoidance of high-light stress, allowing damage to PSII to be redistributed within the leaves (Davis and Hangarter 2012). In response to blue light, CRY1 and CRY2 increase the expression of *psbA* and *psbD* (Thum et al. 2001, Tsunoyama et al. 2004, Onda et al. 2008). These genes promote the production of the D1 and D2 proteins of PSII (Marder et al. 1987) which are needed to repair damage to PSII (Christopher and Mullet 1994). Similarly, UVR8 can induce production of the D2 protein, though not D1 protein in PSII, in response to UV-B radiation (Davey et al. 2012). However, little is known about the roles of UVR8, CRYs and PHOTs in regulating the photosynthetic capacity of PSII in response to UV-A radiation.

We had three major aims in this experiment. Firstly, to test the respective roles of blue and UV-A radiation in the accumulation of flavonoids in plants growing in a low-to-moderate light environment (168 μmol m^{-2} s^{-1} of PAR) and to identify those photoreceptors responsible for this response. Secondly, to determine whether growth under these blue light and UV-A radiation treatments provided pre-emptive acclimation to high-light (priming), and lastly whether flavonoid content was correlated with the protection of PSII. To address these questions, we examined wild type (WT) L*er* and mutants deficient in UVR8, CRY1CRY2 and PHOT1 activity. We hypothesised that UVR8 would mediate an increase in flavonoid accumulation and promote quantum efficiency of PSII in response to UV-A radiation through increased photoprotection. We expected cryptochromes to increase leaf flavonoid content and promote the quantum efficiency of PSII under blue light and UV-A radiation, and for this also to benefit PSII under high-light exposure. Lastly, we considered that phot1 in plants growing under blue light and UV-A radiation might promote cry-mediated anthocyanin accumulation and thus increase the overall phenolic content.

Materials and Methods

Plant Material

All genotypes used in this experiment had the background accession *Arabidopsis thaliana* Landsberg *erecta*. All seeds were produced simultaneously from plants grown under the same standard

conditions in the growth rooms and greenhouses at the University of Helsinki, Viikki campus. The following photoreceptor mutants were included in the experiments: *phot1* (Inada et al. 2004), *cry1 cry2* (Mazzella et al. 2001), and *uvr8-2* (Brown et al. 2005).

Light treatments

The experiment was set up in six individual compartments of equal size (97 cm wide × 57 cm deep × 57 cm high) in a temperature-controlled room. These were arranged into three blocks, with each block containing two compartments, each randomly allocated a light treatment (Blue Light / No Blue Light, hereafter referred to as BL and no BL treatments). This meant that in total three of the six compartments received a broad spectrum of irradiance from an array of LED lamps (Valoya AP67, 400-750nm, PAR 168 µmol m^{-2} s^{-1} plus 32 µmol m^{-2} s^{-1} far red) and in the other three compartments blue light was attenuated by wrapping film Rosco #313 Canary Yellow (Westlighting, Helsinki, Finland) around the LED lamps (no BL): the height of lamps used in either treatment was adjusted to compensate for the lack of blue light and equalise the PAR received across the treatments (SI Appendix 1 Fig. S1, Table S1). All compartments were protected from extraneous light with white-black plastic film that blocked visible and UV radiation. The number of layers of these plastic sheets was also adjusted to help equalise temperature across the treatments (SI Appendix 1 Table S2). The UV-A treatment was applied as a split-plot factor by dividing each of the six compartments in two halves with a curtain of Rosco #226 film (Westlighting, Helsinki, Finland) attenuating all UV-A radiation (λ <400 nm): one half of each compartment received UV-A radiation from high power LED arrays with peak emission at 365 nm (mean and SE of 15.0 ± 0.6 µmol m^{-2} s^{-1}: Z1-Z1-10UV00, LED Engin, San Jose, CA, USA). Spectral irradiance measurements were made in each compartment with an array spectroradiometer (Maya 2000Pro, Ocean Optics Inc., Dunedin, Florida, USA). The BL and UV-A treatments were chosen to approximate the ratio of UV-A: BL and PAR irradiance that we measured at noon in under-canopy shade in a *Betula* stand in southern Finland (Fig. 1, example of under-canopy spectral irradiance, SI Appendix 1 Table S3 and Table S4). The broad-spectrum lamps were kept on a 10-hour photoperiod from 08:00 to 18:00, whereas the UV-A LEDs were kept on for a 4-hour period centred around 'noon' from 10:30 to 14:30, when UV-A irradiance is at its highest in natural sunlight (Flint and Caldwell, 1998).

[Insert Fig. 1]

Growing conditions

Five seeds per 6-×-6 cm pot were sown directly into the growing substrate; well-soaked 1:1 pre-fertilised peat to vermiculite (Agra-vermiculite; Pull Rhenen, TX Rhenen, the Netherlands) – a thin layer

of peat was sieved at 2 mm gauge to provide a smooth surface giving good contact with the germinating seeds. After sowing, pots were placed for one day in darkness at 15°C to allow the seeds to become fully imbibed, then for three days the pots were under the light treatments at 5°C-day / 2°C-night to facilitate cold stratification, before increasing to reasonable spring-time temperature for northern temperate latitudes of 15°C day / 10°C night (± SE 0.24°C), with a VPD of 0.17 kPa day/ 0.18 kPa night during germination and subsequent growth. Temperature was continuously monitored with iButton sensors (Maxim Integrated, San Jose, United States) for the entire duration of the experiment to check that temperature was consistent among all compartments (SI Appendix 1 Table S2).

After germination when the first pair of true leaves became visible, the seedlings were thinned to one plant per pot, leaving 288 plants in the experiment: 6 plants × 5 genotypes × 6 compartments × 2 split with or without UV-A radiation. The soil moisture was monitored daily, and maintained by adding *c* 50 ml per pot every 5-7 days. Plants were rotated within compartments to attenuate any unknown gradients in temperature, relative humidity, and irradiance.

Acute high-light exposure

Plants from each growth condition were randomly separated into two groups, a control group remaining under the growth conditions, and a group which would be exposed to an acute high-light-stress treatment. Each group contained 6 plants per treatment combination (genotype × blue × UV-A × high-light) divided in equal numbers per block.

High-light treatments were made in a temperature-controlled greenhouse compartment at 25°C. The lamps (Osram Powerstar HQI-E 400W/D stadium lamps, Osram GmbH, Munich, Germany) warmed up for at least 1 hr prior to implementing the treatment to ensure their emission was stable, and 75-mm deep baths of flowing water, placed between the lamps and plants, served as a heat sink reducing the warming effect of the lamps. Plants were kept underneath the lamps for exactly 30 min, at which point the quantum yield of PSII was measured before they were returned to their growing conditions. Photon irradiance incident on the leaves of plants receiving the high-light treatment was 1800-2100 $\mu mol^{-1}\ m^{-2}\ s^{-1}$ PAR (SI Appendix 1 Figure S3, Table S5).

Non-invasive measurements of UV-screening and leaf chlorophyll content

A Dualex Scientific^{+} device (Force-A ^{TM}, Paris, France) was used to make non-destructive measurements of leaf pigments based on their optical properties. The leaf chlorophyll content is calculated by the Dualex device based on the difference in transmission through the leaf in the near infra-red at 710 nm and 850 nm (NIR), and in the red regions of the spectrum (Cerovic et al. 2012). The relationship between leaf chlorophyll content ($\mu g\ cm^{-2}$) and the chlorophyll-specific absorption index

measured with the Dualex Scientific^{+} was linear across the range of values we obtained (Robson et al. unpublished data, Parry et al. 2014). The relative leaf adaxial-epidermal UV-A-absorbance at 375 nm is calculated by the Dualex device based on chlorophyll fluorescence at 375 nm relative to chlorophyll fluorescence in the red region of the spectrum while accounting for leaf chlorophyll content (Cerovic et al. 2012). There is a linear relationship between this index of UV-absorbance at 375 nm and epidermal flavonol content for the range of values that we report (Robson et al. unpublished data, also reviewed by Julkunen-Tiitto et al. 2015).

Dualex measurements were made from all plants from both control and high-light groups, 45 days after germination under the growing light conditions of the experiment between 15:30 and 18:00. A second set of Dualex measurements were then taken three hours after the end of the high-light exposure (day 46) back in the experimental compartments of the growth room. Both sets of measurements were made on the same two fully-expanded mature non-senescent unshaded leaves per plant that were horizontal to the light source at the point of measurement. Plants were all rosettes with no visible signs of development beyond their vegetative state at the time of measurement.

Measurements of the quantum yield of PSII

Quantum yield at midday (day 45), of same two leaves per plant selected for Dualex measurements, was measured with a PAM fluorometer (mini-PAM, Heinz-Walz GmbH, Effeltrich, Germany). We used ϕPSII (calculated as $F_q`/F_m`$; Murchie and Lawson, 2013) as an indicator of the operating efficiency of PSII during the day for plants under the light treatments (day 45). It was possible to compare the $F_q`/F_m`$ of leaves measured in ambient conditions under the light treatments since the photon irradiance was matched to be as similar as possible in each treatment combination. A second set of $F_q`/F_m`$ measurements were made the following day (day 46) between 10:00 and 12:00 for the control group under growing light treatments (as before), and between 12:00 and 13:50 for the test group under high-light exposure. To assess damage to PSII, F_v/F_m of all plants was measured from the same leaves as ϕPSII measurements after they had been dark-adapted for at least 30 min on day 45 and on day 46 at 19:00 hrs.

Quantitative Analysis of Soluble Phenolic Compounds

HPLC Analysis

Directly following the Dualex measurements after the high-light exposure (day 46), the leaves used for these optical assessments were harvested from both control and high-light plants. Only those leaves, or parts of leaves, that were unshaded by other leaves in the rosette were selected for biochemical extraction of soluble (i.e. non-cell-wall bound) phenolic compounds. Likewise, the petiole and proximal lamina section were excluded ensuring that the sampled leaves had actually received their respective radiation

treatments, following Julkunen-Tiitto et al. (2015). After each sample was harvested, they were weighed ensuring that there was at least 100 mg fresh weight of each sample, and immediately frozen in liquid nitrogen inside Al-foil packets and stored at -80°C. Samples were lyophilised in the dark and transported on dry ice for HPLC and UV/Vis analyses. Leaves were ground in 3-ml 40% methanol using a pestle and mortar, before being placed in an ultrasonic bath (Ultrasonic compact cleaner UC 006 DM1, Tesla, CZ), and sonicated for 5 min. The samples were then centrifuged for 3 min at 6000 RPM, equivalent of 3461 RCF (EBA 20 Hettich Zentrifugen, Germany). 1 ml of supernatant was filtered through a 0.2 μm filter (Premium Syringe Filters, Agilent, USA) and used for HPLC-DAD analysis with an Agilent 1200 HPLC system (Agilent Technologies, USA). Each extract for HPLC-DAD (1 ml) was collected in a vial, and 5 μl of sample was injected into the HPLC for each analysis. Separation was performed using a Hypersil Gold (C18, 50 x 2.1 mm column, with 1.9 μm particles, Thermofisher scientific, San Jose, CA, USA) chromatographic column which was tempered to 30 °C during the separation process. Two mobile phases were used, mobile phase A consisted of 5% acetonitrile, and mobile phase B consisted of 80% acetonitrile, and both phases were acidified using methanoic acid with a ratio of 1:999 v/v. Flow of the mobile phases was 0.3 ml min^{-1}. Compounds were detected at 270, 314 and 360 nm. For the purpose of quantification of phenolic compounds, and for particular flavonoids, peaks on an optical chromatogram detected at 360 nm were manually integrated. Peak areas were adjusted against the fresh weight of leaves and used to determined relative content of compounds in samples. The spectra of phenolic compounds were measured across a spectral interval of 190-750 nm. The identification of compounds was done with an UltiMate 3000 HPLC system (Thermofisher Scientific, USA San Jose, CA) followed by Q-TOF mass spectrometer (micrOTOF-QII, Bruker Daltonics, Germany) by comparing the retention time order, UV-VIS absorption spectra, and the mass per charge ratio of the mother and fragment ions of each phenolic compound against values from the literature (SI Appendix 2). The compound identity was subsequently assigned to individual peaks detected during quantitative HPLC-DAD analysis according to their retention time and UV-VIS absorption spectra similarity (SI Appendix 2).

Data Analysis

The effects of genotype, blue light, UV-A radiation, and high-light exposure and their interaction were tested using analysis of variance (ANOVA). All statistical tests were done in R version 3.2.2 (2016, The R Foundation for Statistical Computing, Vienna, Austria). Data were analysed using a linear mixed-effects model (LME), which was fitted using the NLME package (Pinheiro and Bates 2000). The function 'weights=varPower' was used to reduce heterogeneity of variance in the model.

A split-plot experimental design was used with BL/no BL the main effect factor, UV-A/no UV-A the split-plot factor, and genotype nested within the split-plot factor. At the end of the experiment, high

light was also included in the model. There were three replicates of each treatment combination for each genotype, corresponding to the number of split-compartments receiving the same treatment.

The six compartments were divided into three blocks based on the arrangement of pairs of BL and no BL treatments. Block was used as a random factor in the model. Multiple comparisons were only tested when treatment effects were $p < 0.05$ using a fit-contrast comparison to test for differences between each genotype and the WT. Holm's correction was used to adjust p values for multiple testing. The R package ggplot2 (Wickham and Chang 2013) was used to construct all figures. Pearson's correlation was used to test the relationship of epidermal flavonoids with ϕPSII and $F_v/F_{m,}$ as well as leaf chlorophyll with ϕPSII and $F_v/F_{m.}$

Results

The accumulation of flavonoids is associated with CRYs under BL but both CRYs and UVR8 under UV-A radiation

Non-invasive measurements of UV-screening

Genotypes differed in their adaxial leaf flavonol content, as assessed by Dualex, (p<0.001, SI Appendix 1 Table S6A). Although the effect of BL was marginally non-significant overall (p=0.062), there was a significant interactive effect of BL and genotype on UV-screening (Genotype × BL: p<0.001). Similarly, while UV-A had no significant effect overall (p=0.217), there was a significant Genotype × UV-A interaction (p= 0.006).

These interactions indicate that the response to each of BL and UV-A radiation differed among genotypes. BL significantly increased UV-screening in *uvr8-2*, *phot1* and WT (respectively p=0.022, p=0.034, p=0.020; Fig. 2), but had no effect compared with no BL in *cry1 cry2* (p=0.430). In the presence of BL, *cry1 cry2* had the lowest epidermal flavonol contents of all the genotypes, and was 24.5% lower than WT (p<0.001, Table 1, Fig. 2).

Considering those treatments without BL, epidermal flavonol content in the upper epidermis of *phot1* plants was lower than that of WT, in both treatments with and without UV-A radiation (p=0.001, p=0.021, respectively). In contrast, *uvr8-2* grown in the absence of both BL and UV-A, had higher leaf epidermal flavonol content than WT (p=0.007), and *cry1 cry2* was not significantly higher than WT (p=0.422, Fig. 2). In the treatment receiving UV-A radiation but not BL, there was no significant difference in the epidermal flavonol content in either *uvr8-2* or *cry1 cry2* compared to the WT (p=0.290, p=0.934).

Growth under the UV-A radiation treatments caused a decrease in epidermal flavonol content compared with no UV-A (irrespective of BL) in *cry1 cry2,* and *uvr8-2* (p<0.002, p=0.022), and had no effect on *phot1* or WT (p=0.931, p= 0.256). After a 4 h period back in their growing conditions, the 30

min of high-light exposure had caused no significant effect on the leaf adaxial epidermal anthocyanin or flavonol content (p=0.895, p=0.305 Fig. 2 & SI Appendix 1 Fig. S3), but leaf chlorophyll content was 6.8% higher in plants exposed to high-light (p=0.026, SI Appendix 1 Fig. S4).

[Insert Fig. 2]

Epidermal anthocyanin content followed the opposite trends to epidermal flavonol content in general, hence in plants with low epidermal flavonol content the epidermal anthocyanin content tended to be high, but a genotype-specific difference in this relationship was not detected (Table 1, SI Appendix 1 Figure S5). This pattern also meant that epidermal anthocyanin content was higher in the no BL than to the BL treatments, however the relative differences between genotypes and treatments were much smaller than for epidermal flavonol content (Table 1, Fig. 2). Leaf chlorophyll content tended to co-vary in the same direction as epidermal flavonoids among genotypes and light treatments (Table 1). All genotypes apart from *cry1cry2* had higher chlorophyll content in the BL treatment than the no BL treatment (Table 1), whereas the effect of UV-A radiation on chlorophyll content was non-significant (SI Appendix 1 Table S6B).

[Insert Table 1]

Qualitative analysis of the effects of blue light and UV-A radiation on leaf phenolic compounds

The same nine phenolic compounds were present in all our treatments at concentrations above the detection limits, allowing their quantification by HPLC-DAD (listed in Table 2). Of these nine compounds, four were confirmed to be kaempferol derivatives, three were sinapic-acid derivatives, and two could not be positively identified.

Genotype had a significant effect overall on the sum of all measured phenolic compounds per unit fresh weight (henceforth, phenolic content; p=0.002, Fig. 3). BL and UV-A did not have a significant effect overall on total phenolic content (p=0.158, p=0.124), however, genotype and BL had a significant interactive effect (p=0.004) following a similar pattern in the direction and size of effects to the leaf adaxial-epidermal flavonol content measured with the Dualex. BL significantly increased total phenolic content in *phot1*, compared with the no BL treatment (p=0.012, Fig. 3, Table 2), but had no significant effect on any other genotypes (Table 2). Pairwise comparisons of genotypes revealed that in the BL only *cry1 cry2* had significantly lower total phenolic content than WT (p<0.001), but no other significant differences between genotypes and WT (Fig. 3, SI Appendix 1 Table S7). High-light treatment had no significant effect on the total phenolic content of leaves sampled 4 h after the exposure (SI Appendix 1

Table S8A-K).

[Insert Fig. 3]

The response to growth under BL of the content of total kaempferol derivatives per unit fresh weight was dependent on genotype, given that genotype had a significant effect overall (p=0.001), and there was a significant interaction between genotype and BL (p=0.027: Table 2). However BL and UV-A radiation had no significant overall effect on kaempferol content (p=0.161, p=0.094). Pairwise comparisons also showed that when *cry1 cry2* was grown under BL, it had 11.4% lower total kaempferol content than WT (p=0.040: Table 2), but *phot1* and *uvr8-2* did not differ significantly from WT (p=0.097, p=0.453). The accumulation of kaempferol derivatives appeared to be partly regulated by phot1 in the absence of BL, as only *phot1* had a significantly lower kaempferol content than WT in the no BL; a difference of 12%.

As with kaempferol derivatives, the concentration of the total sinapic-acid derivatives per unit fresh weight differed among genotypes (p<0.001). BL had no significant effect overall on sinapic-acid content (p=0.154), but this was because the genotypes responded in contrasting ways to BL (significant interactive effect; p<0.001). UV-A radiation had no significant overall effect on sinapic-acid content (p=0.210). Under the BL treatment, *phot1* mutants had a significantly higher total sinapic-acid content than without BL (p=0.023), but the other genotypes did not differ (Table 2). Pairwise comparisons of sinapic-acid contents between the WT and the photoreceptor mutants, showed that under BL leaves of *cry1 cry2* and *phot1* had a significantly lower content of sinapic-acid derivatives than those of WT (p<0.001, p=0.012). In the no BL treatment, *phot1* had significantly lower sinapic-acid content than WT (p=0.006), with all other genotypes showing no significant differences from WT (Table 2).

Among the individual phenolic compounds identified, genotype had a significant effect on the concentrations of sinapoyl glucose (p<0.001), sinapoyl tartronate (p<0.001), kaempferol 3-O-rha-glu 7-O-rha (p<0.001), kaempferol 3-O-glu-glu 7-O-rha (p=0.002) and kaempferol 3-O-glu 7-O-rha (p=0.014). The response of these compounds to UV-A, and the interaction between BL and genotype, were consistent in their direction and extent with the responses of the total content of phenolic compounds described above (Table 2, SI Appendix 1 , Table S7). Although we report differences in the content of these various phenolic compounds among our treatments, as described above, we did not detect a change in the composition of phenolic compounds between treatments.

[Insert Table 2]

Blue light increases the quantum yield of photosystem II via CRYs under growth conditions

To investigate the role of each class of photoreceptors in maintaining PSII function, we first measured ϕPSII, and the maximum quantum yield (F_v/F_m) of dark-adapted plants, under their growing conditions to serve as a baseline to compare against ϕPSII under high light and F_v/F_m after high-light exposure.

The F_v/F_m of dark-adapted plants under their growing conditions differed significantly with genotype (p=0.012) and BL (p=0.049), but not UV-A (p=0.314). Genotypes differed in their response of F_v/F_m to BL, producing a significant interaction between genotype and BL (p=0.036). BL significantly increased F_v/F_m compared with no BL in WT and *phot1* (p=0.037, p=0.030), but had no significant effect on *cry1 cry2* nor *uvr8-2* (p=0.301, p=0.109). This result meant that *cry1 cry2* had a significantly lower F_v/F_m than WT under the BL treatment (p<0.001), while the other genotypes were not significantly different from the WT (Fig. 4).

[Insert Fig. 4]

[Insert Fig. 5]

Genotype and BL also significantly affected ϕPSII under growth conditions (p<0.001, p=0.010, Fig. 5); UV-A had no significant effect (p=0.169). BL significantly increased ϕPSII in *phot1*, *uvr8-2* and WT (p=0.028, p=0.016, p=0.031), but had no significant effect on *cry1 cry2* (p=0.215). In the BL treatment, *cry1 cry2* had the lowest ϕPSII of all genotypes (p<0.001), but no other genotypes were significantly different from WT (Fig. 5). When grown in the absence of BL, *phot1* had significantly lower ϕPSII in comparison to WT (p=0.001), however there were no other significant differences from the WT among genotypes in the no BL treatment (Table S9).

[Insert Fig. 6]

[Insert Fig. 7]

Under 30-min saturating high-light treatment, ϕPSII was reduced, but also became more variable within genotypes and treatment combinations, compared with plants not receiving high light. This may partially explain why, although ϕPSII under high light differed with genotype (p<0.001), previous growth under BL or UV-A had no significant overall effect, nor interactive effects with genotype (Fig. 6; Table 1). Among genotypes, *cry1 cry2* had the lowest ϕPSII under high light and was the only mutant to significantly differ from the WT (p=0.007). The F_v/F_m measured in dark-adapted leaves 4 hrs after the end

of the acute high-light exposure was not significantly affected by genotype, BL nor UV-A (Fig. 7, p=0.080, p=0.234, p=0.338, respectively). Electron transport rate (ETR) and non-photochemical quenching (NPQ) were higher in all genotypes under the high-light exposure, but were not significantly different between genotypes or treatments (SI Appendix 1, Fig S17 and FigS18).

Discussion

Cryptochromes and UVR8 are both associated with photosynthetic-pigment accumulation in response to low UV-A radiation treatments during growth.

Our experiment examined how growth under broad spectrum irradiance treatments with and without BL and UV-A radiation affected photoprotection and flavonoid accumulation. Under these conditions, based on a ratio of UV-A:BL similar to that in understorey shade, BL had a relatively large effect compared with UV-A on leaf epidermal UV-screening by flavonoids. This flavonoid response to BL can be largely attributed to the role of cryptochromes. Within each genotype, the effects of BL and UV-A radiation on phenolic content quantified from leaf extracts were consistent with those for UV-epidermal screening by flavonols assessed optically, however among genotypes this relationship was not as consistent (Table 2, Figs. 2 & 3). Considered together, the increase in optically-assessed flavonol content in plants grown under BL, as well as several phenolic compounds quantified from leaf extracts, can be attributed to the role of cryptochromes in promoting the accumulation of phenolic compounds in response to blue light.

[Insert Fig. 8]

Recent studies reporting large increases in the accumulation of phenolic compounds and flavonoids caused by blue light have been conducted outdoors in experiments compared against attenuated solar blue light (Siipola et al. 2015), or in controlled conditions using high irradiances of blue light, together with PAR equivalent to full sunlight (Hoffman et al. 2015, Taulavuori et al. 2016). However, even with the moderate PAR in our experiment, our BL treatment had a strong effect on phenolic accumulation which outweighed that of our UV-A radiation treatment. From our results, we can assert that functional CRY1 and CRY2, promote photoprotective, as well as photomorphogenic responses to blue light (Vandenbussche et al. 2005, Keller et al. 2011). Similarly, the small increase we report in chlorophyll content in response to BL can also be attributed to the role of functional CRYs (Table 1). CRY 1 has been reported to maintain a high concentration of chlorophyll in *A. thaliana* seedlings exposed to 24 hrs high light (Kleine et al. 2007), and to increase chlorophyll concentration in the roots of *A. thaliana* in response

453 to blue light (Usami et al. 2004). Surprisingly, our results are the first that we are aware of to find that
454 CRYs mediate an increase in chlorophyll content in the leaves of *A. thaliana* grown under blue light.
455 Although it has already been shown that phot1 promotes cry-mediated accumulation of
456 anthocyanins (Kang et al. 2008), hitherto there was no evidence that phot1 elicits the accumulation of
457 flavonoids in the absence of blue light. Our result, that epidermal flavonol content was significantly lower
458 in *phot1* when BL was attenuated, suggests that phot1 may contribute to the baseline accumulation of
459 flavonoids in the absence of (or at very low irradiances of) blue light. This does not necessarily imply that
460 phot1 itself promotes pathways that can mediate flavonoid accumulation, maybe phot1 simply affects the
461 metabolism of the plant in such a way that directs investment into the production of flavonoids. In this
462 respect, it would be interesting to know how phot interacts with other photoreceptors to regulate
463 constitutive flavonoid production.
464 We hypothesized that UV-A radiation would increase flavonol content in our WT plants based on
465 the results of previous solar UV-attenuation studies outdoors which differentially-attenuated UV-A
466 radiation from the spectrum (Ibdah et al. 2002 [attenuated 50% solar <360 nm], Kotilainen et al. 2008,
467 Kotilainen et al. 2009, Morales et al. 2013 [all three attenuated 50% solar <400nm]). However, our UV-A
468 treatment did not increase epidermal flavonol content assessed using Dualex. There are several possible
469 explanations for this discrepancy between our results and UV-A supplementation studies. We used a UV-A
470 LED source with a narrow peak at 365 nm, whereas the majority of UV-A-supplementation studies which
471 have used UV-A fluorescent lamps with a broader spectrum across the UV-A region (Joshi et al. 2007
472 [330-390 nm], Victório et al. 2011 [320-400 nm], Štroch et al 2015 [350-400 nm]). The absorption spectra
473 of all the photoreceptors considered here, and additionally phytochrome, absorb in the UV-A region
474 (Shinomura et al. 1996, Briggs and Huala 1999, Heijde and Ulm, 2012). This potential for interactions
475 among photoreceptors may imply that responses elicited are highly wavelength dependent within the UV-
476 A region. Alternatively, it is possible that the plants acclimated to the low but ecologically-realistic UV-A
477 irradiance used in this experiment (15 μmol m^{-2} s^{-1}: Fig. 1, SI Appendix 1 Tables S1, S2, S3) throughout
478 their growth, or that it was not high enough to elicit a flavonoid response in WT. Transmittance to the
479 chloroplasts of non-damaging amounts of UV-A radiation may even be useful since it can drive
480 photosynthesis (Bilger et al. 2007, Turnbull et al. 2013, Štroch et al 2015), being absorbed by chlorophyll
481 and inducing chlorophyll fluorescence (McCree 1981, Lang and Lichtenthaler 1991). The potential
482 benefits of UV-A radiation for photosynthesis could be more pronounced in shaded understorey
483 conditions, where light is often limiting and the UV-A irradiance is considered unlikely to be high enough
484 to induce stress or photodamage (Štroch et al 2015 Casal 2013b) but is proportionally enriched compared
485 to PAR (Flint and Caldwell 1998, Leuchner et al. 2011, Urban et al. 2012, Dengel et al. 2015).
486 Although PHOT1 absorbs UV-A radiation, and has been shown to have an action spectrum for

tropism in the UV-A region, our UV-A treatment did not affect either the epidermal flavonol content or leaf phenolic content of *phot1* mutants. It has previously been suggested that the flavonoid response to BL and UV-A radiation in *A. thaliana* is most likely to be largely driven by CRY1 and CRY2, which control CHS expression and the subsequent flavonoid biosynthesis (Jenkins et al. 2001). Our results provide evidence that cryptochromes and UVR8 (but not phototropin1) are both involved in eliciting flavonoids in response to UV-A radiation. Whilst UVR8 has an absorption tail to at least 315nm (Christie et al. 2012), there is currently no evidence of absorption by UVR8 as far as 365nm; the UV-A peak emitted by LEDs in our experiment. However, other studies have shown UVR8 to affect the accumulation of flavonoids in response to solar UV-A (Morales et al. 2013). An in-depth study of how far into the UV-A region UVR8 is able to coordinate responses would be of great interest.

It is possible that pleiotropic effects could contribute to the flavonoid response to BL and UV-A among the different genotypes used in our experiment e.g. the mutations could indirectly affect photosynthesis and other metabolic pathways that could also contribute to differences in plant physiology including flavonoids. However, for cryptochromes and UVR8 there is already some evidence from studies of gene expression, as well as transcripts and metabolites suggesting their regulation of flavonoids in response to BL and UV-A radiation (Wade et al. 2001, Morales et al. 2013), whereas such evidence is lacking for phot1.

Cryptochromes promote accumulation of soluble leaf phenolic compounds in response to both blue light and UV-A radiation, whereas UVR8 promotes accumulation of sinapoyl glucose in response to UV-A radiation

The composition of phenolic compounds, as opposed the total phenolic content, is often reported to change in response to blue light and UV-A radiation (Morales et al. 2010, Morales et al. 2013, Siipola et al. 2015). A coherent change in composition would involve the conversion of kaempferols to quercetin-derivatives which have higher ROS-scavenging activity, along with sinapic-acid derivatives such as sinapate esters, which absorb more UV radiation in epidermal cells (Sheahan, 1996, Götz et al. 2010, Agati et al. 2012, Csepregi et al. 2016). In contrast to this expectation, our treatments did not produce detectable changes in the composition of kaempferol and sinapic-acid derivatives, only affecting their content. The directional effects of BL and UV-A on leaf phenolic content were consistent with the changes in epidermal flavonoid content (Figs. 2 & 3), although to varying degrees of significance (SI Appendix 1 Table S6A, S7 and Tables S8A-K, Fig S15 and S16). Such a result is consistent with studies that report a change in the total content of phenolic compounds in response to UV-A, as opposed to a change in their composition (Maffei et al. 1999, Lee et al. 2014). Cryptochromes have been reported to mediate a general increase in flavonoid accumulation in response to blue light (Ouzounis et al. 2015, Taulavuori et al. 2016),

and blue light can have species-specific effects on the total concentration of phenolic-acid derivatives (Ouzounis et al. 2015, Taulavuori et al. 2016). There has been to-date little evidence of a specific contribution of cryptochromes to the accumulation of particular groups of phenolic compounds in response to BL as well as to UV-A radiation. Here, we found that cryptochromes were associated with the accumulation of total phenolic-acid derivatives, several individual sinapic-acid derivatives and kaempferol derivatives in response to growth under BL, as well as the accumulation of sinapoyl glucose and kaempferol 3-O-glu-glu-7-O-rha in response to growth under UV-A radiation.

In addition to cryptochromes, functional UVR8 promoted the accumulation of sinapoyl glucose as well as epidermal flavonols in response to UV-A radiation. This result supports the suggestion by Morales et al. (2013) that cryptochromes and UVR8 could modulate the accumulation of phenolic compounds in the UV-A region. One potential reason for plants to have multiple photoreceptors whose absorption spectra lie within the UV-A region is that coordination between crys and UVR8 could provide the optimal composition of phenolic compounds for growth and defense in a dynamic light environment where the ratio of UV radiation to blue light often differs from that of full sunshine (Fig. 1, SI Appendix 1 Table S3). However, we are not yet in a position to identify the signalling mechanisms by which cryptochromes and UVR8 could co-regulate the accumulation of phenolic compounds in general, nor the interconversion of specific compounds from the phenolpropanoid pathway (Wade et al. 2001, Morales et al. 2013).

Moderate blue light but not UV-A radiation during growth enhances the operating efficiency and maximal quantum yield of PSII via cryptochromes

The UV-A treatment used in our experiment did not have any significant effect on the operating efficiency of PSII (ϕPSII) nor on the maximum quantum efficiency of PSII (F_v/F_m). Similarly, a study using higher UV-A irradiance on barley (*Hordeum vulgare*) found no significant effect on F_v/F_m (Štroch et al. 2015). However, under unshaded conditions solar UV-A radiation has been reported to decrease F_v/F_m in the *A. thaliana* mutant *uvr8-1* (Coffey et al. 2017). Increases in F_v/F_m for plants that have been cultivated under blue light have been reported in many plant species (Goins et al. 1997, Matsuda et al. 2008, Terfa et al. 2013*,* Hoffmann et al. 2015), yet to our knowledge this is the first study of the effects of blue light on ϕPSII and F_v/F_m to compare differences amongst *A. thaliana* photoreceptor mutants *cry1 cry2, phot1* and *uvr8-2*. Here, we report that CRYs mediated an increase of both ϕPSII and F_v/F_m during growth under BL, and ϕPSII when subjected to acute high light, suggesting that CRYs play an important role in the induction of photoprotection. It is conceivable that such a mechanism would be useful for a plant during the transition from shade to a sunfleck, which is accompanied by a particularly large shift in blue light. It has been suggested that CRYs could affect leaf morphology in response to blue light in such a way as to moderate the absorption of light passing through the leaf or alter the ratio of absorption

between PSI and PSII reaction centres (Murchie and Lawson 2013, Miao et al. 2016). Mechanisms such as these may explain the small increase in F_v/F_m and ϕPSII induced by growth under BL in our experiment. CRYs mediate blue-light activation of D1 and D2 proteins, increasing the amount available for PSII repair (Thum et al. 2001, Tsunoyama et al. 2004, Onda et al. 2008), and so may also be integral to the normal functioning of PSII. Such mechanisms could explain the manner by which CRYs increased ϕPSII and F_v/F_m under BL during growth in our experiment.

Klem et al. (2015) found the quantum efficiency of PSII to be positively correlated with leaf flavonoid content in *H. vulgare* under high-light stress. However, in our study there was no overall relationship between ϕPSII or F_v/F_m and with either epidermal UV-screening by flavonoids (SI Appendix 1 Fig. S7- Fig. S10) or leaf chlorophyll content (Fig. S11- S14). These results seem to rule out flavonoids as being directly responsible for an improvement in photoprotection in our experiment. We did not measure the carotenoid content in our samples, however of the induction of carotenoids represents an alternative mechanism whereby plants can mitigate photoinhibition through NPQ (Horton et al. 1996, Li et al. 2000), and may explain those differences in ϕPSII and F_v/F_m among the genotypes that are not consistent with flavonoid accumulation. Boccalandro et al. (2012) reported a reduced photosynthetic capacity of *cry1 cry2* mutants when exposed to high light suggesting that nonstomatal limitations could be responsible: one such limitation is a reduced electron transport rate per unit area (Boonman et al. 2009). Additionally, Kleine et al. (2007) have demonstrated that cry1 is necessary for the activation of 77 genes out of 996 that respond to high light, including the gene for Vitamin B6 which provides antioxidant activity against ROS stress. All of the above examples and our own results support the contribution of CRYs in initiating those high-light responses in plants, through which a higher ϕPSII and F_v/F_m can be maintained through mechanisms besides photoprotection endowed by flavonoids.

Ecological implications of blue light and UV-A photoreceptor responses

Whilst *A. thaliana* is not an understorey species, as a model plant it has provided insight as to how UVR8 and phytochromes coordinate shade responses (Fraser et al. 2016), as well as optimising flavonoid content beneath a patchy canopy (Mazza and Ballaré 2015). Under controlled conditions, we report important roles for cryptochromes and UVR8 in coordinating the accumulation of phenolic compounds in response to UV-A radiation and blue light at an irradiance that can be found in understorey shade. It remains to be seen whether naturally-occurring forest species would respond similarly to equivalent changes in these wavelength regions.

In our experiment, there was a larger effect of blue light increasing flavonoid accumulation than that of UV-A radiation. Although the UV-B irradiance in understorey shade is very low, both UV-A and UV-B radiation are enriched relative to PAR. There is evidence that the photoprotection endowed by

589 flavonoids, especially against high irradiances of UV-B as well as UV-A radiation (Li et al. 1993, Jansen
590 et al. 1998), could constitute a precautionary response to sunflecks by increasing antioxidant capacity
591 (Gould et al.2000). Flavonoid responses to UV radiation are known to be dose dependent (reviewed by
592 Robson et al. 2015). For instance, Morales et al. (2010) reported a dose-dependent response of certain
593 flavonoids to UV-A radiation, which was quadratic under UV-B radiation. Considering the enrichment in
594 UV-B radiation relative to PAR in understorey shade (Flint and Caldwell 1998), the effects of understorey
595 UV-B radiation on flavonoid content and its synergistic effects with blue light would be worthy of
596 investigation since they could be more pronounced than those of UV-A radiation.
597 The epidermal flavonol contents we report were within the range of published values for *A.*
598 *thaliana* exposed to solar radiation for 36 hrs (Morales et al. 2013), but lower than the range of values
599 reported in *H. vulgare* exposed to solar radiation at a higher irradiance of supplemental UV-A than used in
600 our experiment (Klem et al. 2015). The relatively low values of epidermal UVA-absorption in our study
601 maybe one reason we did not find the same correlative relationship between flavonoid content and ϕPSII
602 or F_v/F_m as reported previously by Klem et al. (2015). Although equally, this could reflect species-specific
603 difference in this response for *H. vulgare* and *A. thaliana,* as further exemplified by the cultivar-specific
604 differences in *Lactuca sativa* (Ouzounis et al. 2015). These inconsistencies among species exemplify the
605 need to study the ecological relevance of this response for understorey species, and the physiological
606 mechanisms which allow plants to acclimate and capitalise on transient sunflecks between periods of
607 shade in the understorey.
608
609 **Conclusions**
610 Cryptochromes and UVR8 both have regulatory effects on the flavonoid response to UV-A
611 radiation in plants grown under controlled conditions, in light treatments based on a ratio of UV-A: BL
612 and PAR irradiance measured in understorey shade. Flavonol content in the adaxial epidermis did not
613 significantly correlate with ϕPSII or F_v/F_m values, nor did the UV-A radiation and BL treatments
614 noticeably prime leaves to cope with acute high-light. However, it is clear from our results that
615 cryptochromes are required for plants to attain high ϕPSII and F_v/F_m in the presence of blue light during
616 growth, and to maintain a high ϕPSII under high-light exposure. The decreased tolerance to high light in
617 the *cry1 cry2* mutant (but not in the *uvr8-2* mutant), with no concurrent decrease in epidermal flavonol
618 content, underlines the importance of cryptochromes in acclimation to high light through mechanisms
619 besides photoprotection by flavonoid accumulation.
620
621 **Author Contributions**
622 Craig Brelsford designed and carried out the experiment, analysed the data and wrote the

manuscript. Luis Morales contributed to the writing of and ideas behind the manuscript. Jakub Nezval carried out HPLC analysis, UV-Vis spectrophotometry and contributed to the writing of the manuscript. Titta Kotilainen advised on the experimental design, spectral irradiance measurements, manuscript writing and analysis. Saara Hartikainen helped design, set-up and the sampling of the experiment. Pedro Aphalo helped to interpret the results and write the manuscript. T. Matthew Robson proposed the original experiment, supervised the work and contributed throughout the entire process.

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Figure and Table Legends

Table 1. Chlorophyll content, upper epidermal anthocyanin content, and absorption at 375nm detected spectrophotometrically for different *A. thaliana* genotypes grown under Blue Light (BL) / No Blue Light (No BL) treatments in a split-plot with UV-A, or without UV-A radiation. Chlorophyll and anthocyanin content measured with Dualex. Mean ± 1 standard error.

Table 2. The concentration of total phenolic compounds, total kaempferol derivatives, total sinapic-acid derivatives, sinapoyl glucose, sinapoyl tartronate, kaempferol 3-O-rha-glu-7-O-rha, kaempferol 3-O-glu-glu-7-O-rha, kaempferol 3-O-glu 7-O-rha, sinapoyl malate and kaempferol 3-O-rhamnoside 7-O-rhamnoside. Means (± 1 SE) are displayed for four *A. thaliana* genotypes grown under Blue Light (BL) / No Blue Light (No BL) treatments in a split-plot with UV-A, or without UV-A radiation. Measurements were taken on day 46 after germination. The content of phenolic compounds is given as the integrated peak area per FW mg^{-1}. Mean ± 1 standard error.

Fig. 1. Spectral photon irradiance on 4th June 2016 at solar noon in the understorey of a *Betula* stand at Lammi Biological Station, Finland (130 masl, 61°03'14.3"N 25°02'14.2"E). Irradiance measured in a sunfleck (solid line) is compared with shade caused by canopy foliage (dashed line).

Fig. 2. Epidermal flavonol content estimated through epidermal UV-absorbance at 375 nm with Dualex. Means (± 1 SE) are displayed for four *A. thaliana* genotypes grown under Blue Light (BL) / No Blue Light (No BL) treatments in a split-plot with UV-A (**Δ**) or without UV-A (○) radiation.

Fig. 3. Integrated area of the sum of total phenolic compounds per fresh weight in mg. Means (± 1 SE) are displayed for four *A. thaliana* genotypes grown under Blue Light (BL) / No Blue Light (No BL) treatments in a split-plot with UV-A (**Δ**) or without UV-A (○) radiation. Measurements were taken on day 46 after germination.

Fig. 4. Maximum quantum yield of PSII (F_v/F_m) measured in dark-adapted leaves. Means (± 1 SE) are displayed for four *A. thaliana* genotypes grown under Blue Light (BL) / No Blue Light (No BL) treatments in a split-plot with UV-A (**Δ**) or without UV-A (○) radiation. Measurements were taken under growth conditions.

Fig. 5. Operating efficiency of PSII measured as ϕPSII. Means (± 1 SE) are displayed for four *A. thaliana*

genotypes grown under Blue Light (BL) / No Blue Light (No BL) treatments in a split-plot with UV-A (**Δ**) or without UV-A (○) radiation. Measurements were taken under growth conditions.

Fig. 6. Operating efficiency of PSII measured as ϕPSII. Means (± 1 SE) are displayed for four *A. thaliana* genotypes grown under Blue Light (BL) / No Blue Light (No BL) treatments in a split-plot with UV-A (**Δ**) or without UV-A (○) radiation. Measurements were taken under high-light conditions.

Fig. 7. Maximum quantum yield of PSII (F_v/F_m) measured in dark-adapted leaves. Means (± 1 SE) are displayed for four *A. thaliana* genotypes grown under Blue Light (BL) / No Blue Light (No BL) treatments in a split-plot with UV-A (**Δ**) or without UV-A (○) radiation. Measurements were taken after high-light conditions.

Fig. 8. A schematic representing the effects of low to moderate irradiance of blue light and UV-A radiation on the accumulation of epidermal flavonoids, and the effect of blue light and high light treatments on the maximal quantum efficiency (Fv/Fm) and operational efficiency of PSII (ϕPSII) represented by the image of PSII. Both cryptochromes and UVR8 contribute to the accumulation of epidermal flavonoids in response to UV-A, whereas in response to blue light, cryptochromes enhance the accumulation of epidermal flavonoids.

1041 Table 1

Genotype	LED	UV-A	UV/Vis Absorption Spectrophotometry	Chlorophyll Content	Anthocyanin Content
phot1	BL	No UV-A	17.22 ± 1.02	19.94 ± 0.01	0.180 ± 0.003
cry1 cry2	BL	No UV-A	18.21 ± 1.25	24.25 ± 0.01	0.185 ± 0.004
uvr8-2	BL	No UV-A	21.97 ± 1.48	22.85 ± 0.01	0.187 ± 0.003
WT	BL	No UV-A	20.48 ± 1.60	25.19 ± 0.01	0.175 ± 0.003
phot1	BL	UV-A	16.83 ± 1.89	18.15 ± 0.02	0.180 0.004
cry1 cry2	BL	UV-A	15.35 ± 2.27	24.23 ± 0.01	0.198 ± 0.004
uvr8-2	BL	UV-A	15.32 ± 2.03	24.71 ± 0.01	0.174 ± 0.003
WT	BL	UV-A	19.99 ± 2.61	23.94 ± 0.01	0.173 ± 0.003
phot1	No BL	No UV-A	10.84 ± 1.45	19.45 ± 0.01	0.194 ± 0.004
cry1 cry2	No BL	No UV-A	14.84 ± 1.17	16.62 ± 0.01	0.196 ± 0.005
uvr8-2	No BL	No UV-A	19.13 ± 1.89	20.41 ± 0.01	0.198 ± 0.005
WT	No BL	No UV-A	14.62 ± 0.85	20.44 ± 0.01	0.191 ± 0.003
phot1	No BL	UV-A	9.22 ± 1.03	18.18 ± 0.01	0.198 ± 0.005
cry1 cry2	No BL	UV-A	12.48 ± 1.30	17.85 ± 0.01	0.205 ± 0.007
uvr8-2	No BL	UV-A	14.23 ± 0.80	19.72 ± 0.01	0.192 ± 0.004
WT	No BL	UV-A	13.40 ± 0.89	20.74 ± 0.01	0.189 ± 0.003

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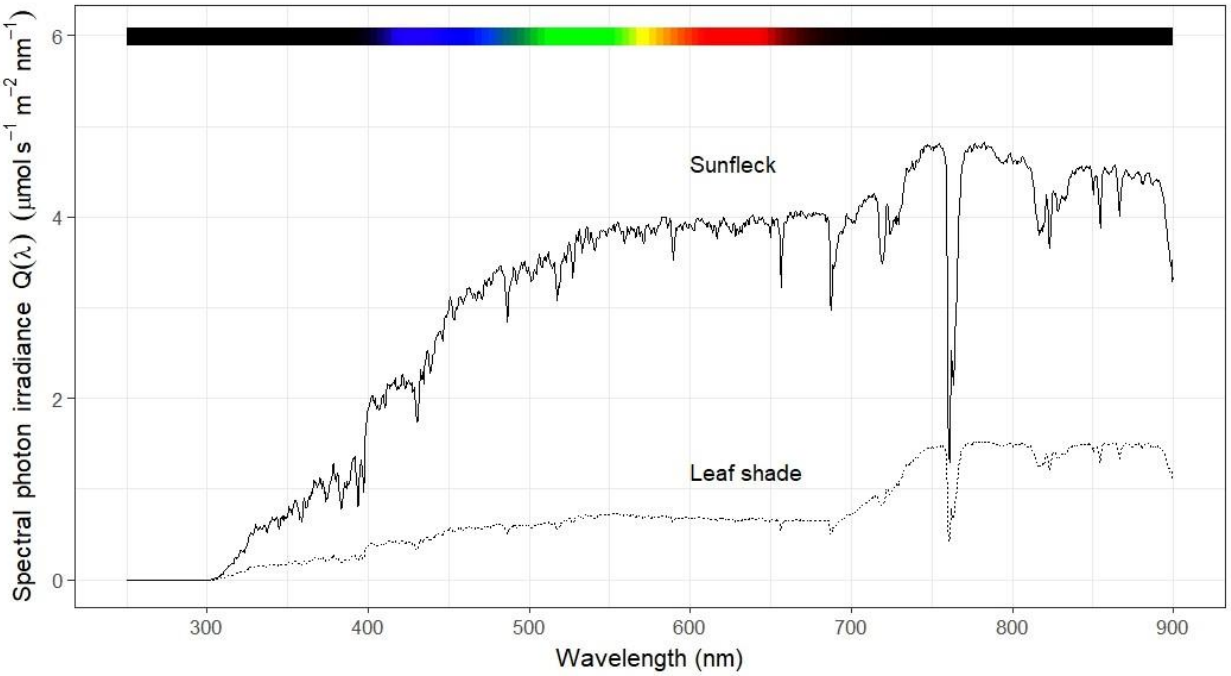
Table 2

LED	UVA	Genotype	total phenolic compounds	total kaempferols	total phenolic acids	sinapoyl glucose	sinapoyl tartronate	kaempferol 3-O-rha-glu-7-O-rha	kaempferol 3-O-glu-glu-7-O-rha	kaempferol 3-O-glu 7-O-rha	sinapoyl malate	Kaempferol 3-O-rhamnoside 7-O-rhamnoside
FullSpec	NoUVA	*phot1*	52.72 ± 5.04	25.96 ± 2.40	17.35 ± 0.94	5.88 ± 0.45	5.91 ± 0.46	5.64 ± 0.25	6.44 ± 0.58	6.50 ± 0.60	6.95 ± 0.70	6.34 ± 0.76
FullSpec	NoUVA	*cry1 cry2*	54.91 ± 1.98	24.02 ± 0.90	18.62 ± 0.66	6.18 ± 0.32	6.29 ± 0.29	6.23 ± 0.30	6.12 ± 0.22	5.98 ± 0.23	6.15 ± 0.33	5.67 ± 0.36
FullSpec	NoUVA	*uvr8-2*	64.73 ± 4.24	28.91 ± 1.86	21.35 ± 1.43	7.28 ± 0.51	7.19 ± 0.50	6.90 ± 0.39	7.16 ± 0.47	9.79 ± 0.48	6.87 ± 0.43	7.21 ± 0.43
FullSpec	NoUVA	WT	61.61 ± 3.55	26.81 ± 1.33	20.88 ± 1.34	7.21 ± 0.55	7.24 ± 0.54	7.09 ± 0.49	6.77 ± 0.39	6.67 ± 0.35	6.43 ± 0.32	6.28 ± 0.23
FullSpec	UVA	*phot1*	40.74 ± 5.58	20.10 ± 2.35	14.85 ± 1.95	4.87 ± 0.76	4.79 ± 0.71	4.88 ± 0.69	5.04 ± 0.63	5.04 ± 0.59	5.19 ± 0.52	5.14 ± 0.48
FullSpec	UVA	*cry1 cry2*	50.96 ± 3.50	22.48 ± 3.28	17.17 ± 1.20	5.69 ± 0.54	5.74 ± 0.51	6.59 ± 1.01	5.67 ± 0.38	6.31 ± 0.81	6.28 ± 0.69	6.11 ± 0.79
FullSpec	UVA	*uvr8-2*	58.23 ± 6.85	26.25 ± 3.09	19.07 ± 2.24	6.19 ± 0.72	6.22 ± 0.73	6.33 ± 0.72	6.48 ± 0.76	6.70 ± 0.79	6.65 ± 0.86	6.13 ± 0.86
FullSpec	UVA	WT	59.49 ± 8.00	25.69 ± 3.69	20.36 ± 2.63	7.23 ± 0.84	7.08 ± 0.80	6.91 ± 0.84	6.50 ± 0.89	8.08 ± 0.88	6.05 ± 1.00	5.94 ± 1.23
NoBlue	NoUVA	*phot1*	47.52 ± 3.37	21.18 ± 1.43	15.86 ± 1.21	5.19 ± 0.45	5.14 ± 0.44	5.20 ± 0.41	5.32 ± 0.38	5.33 ± 0.37	5.53 ± 0.45	5.34 ± 0.39
NoBlue	NoUVA	*cry1 cry2*	39.17 ± 5.50	15.85 ± 3.07	12.58 ± 1.56	3.52 ± 0.35	3.74 ± 0.37	3.91 ± 0.38	4.52 ± 0.63	4.66 ± 0.73	5.33 ± 1.07	3.83 ± 0.83
NoBlue	NoUVA	*uvr8-2*	49.41 ± 5.03	21.31 ± 2.07	16.72 ± 1.76	6.19 ± 0.69	6.09 ± 0.67	5.90 ± 0.64	5.35 ± 0.56	5.27 ± 0.56	4.44 ± 0.41	5.11 ± 0.44
NoBlue	NoUVA	WT	48.27 ± 13.20	21.84 ± 2.15	15.84 ± 1.40	5.14 ± 0.42	5.17 ± 0.43	5.16 ± 1.85	5.33 ± 0.49	6.66 ± 0.53	6.19 ± 0.80	6.29 ± 0.69
NoBlue	UVA	*phot1*	40.15 ± 3.79	18.29 ± 1.73	13.32 ± 1.35	4.04 ± 0.51	4.18 ± 0.49	4.39 ± 0.46	4.52 ± 0.41	4.50 ± 0.45	5.10 ± 0.66	4.74 ± 0.56
NoBlue	UVA	*cry1 cry2*	31.66 ± 2.97	13.46 ± 4.31	9.52 ± 1.02	3.45 ± 0.31	3.70 ± 0.31	3.30 ± 0.22	3.34 ± 0.33	4.37 ± 0.31	3.73 ± 0.32	3.73 ± 1.95
NoBlue	UVA	*uvr8-2*	40.13 ± 2.35	17.31 ± 1.09	13.67 ± 0.75	4.89 ± 0.27	4.77 ± 0.27	4.73 ± 0.28	4.37 ± 0.28	4.31 ± 0.29	3.94 ± 0.24	3.91 ± 0.28
NoBlue	UVA	WT	38.01 ± 3.25	16.74 ± 1.65	12.77 ± 0.99	4.36 ± 0.34	4.32 ± 0.33	4.24 ± 0.35	4.20 ± 0.38	4.18 ± 0.40	4.09 ± 0.48	4.13 ± 0.65

1049
1050 Fig. 1



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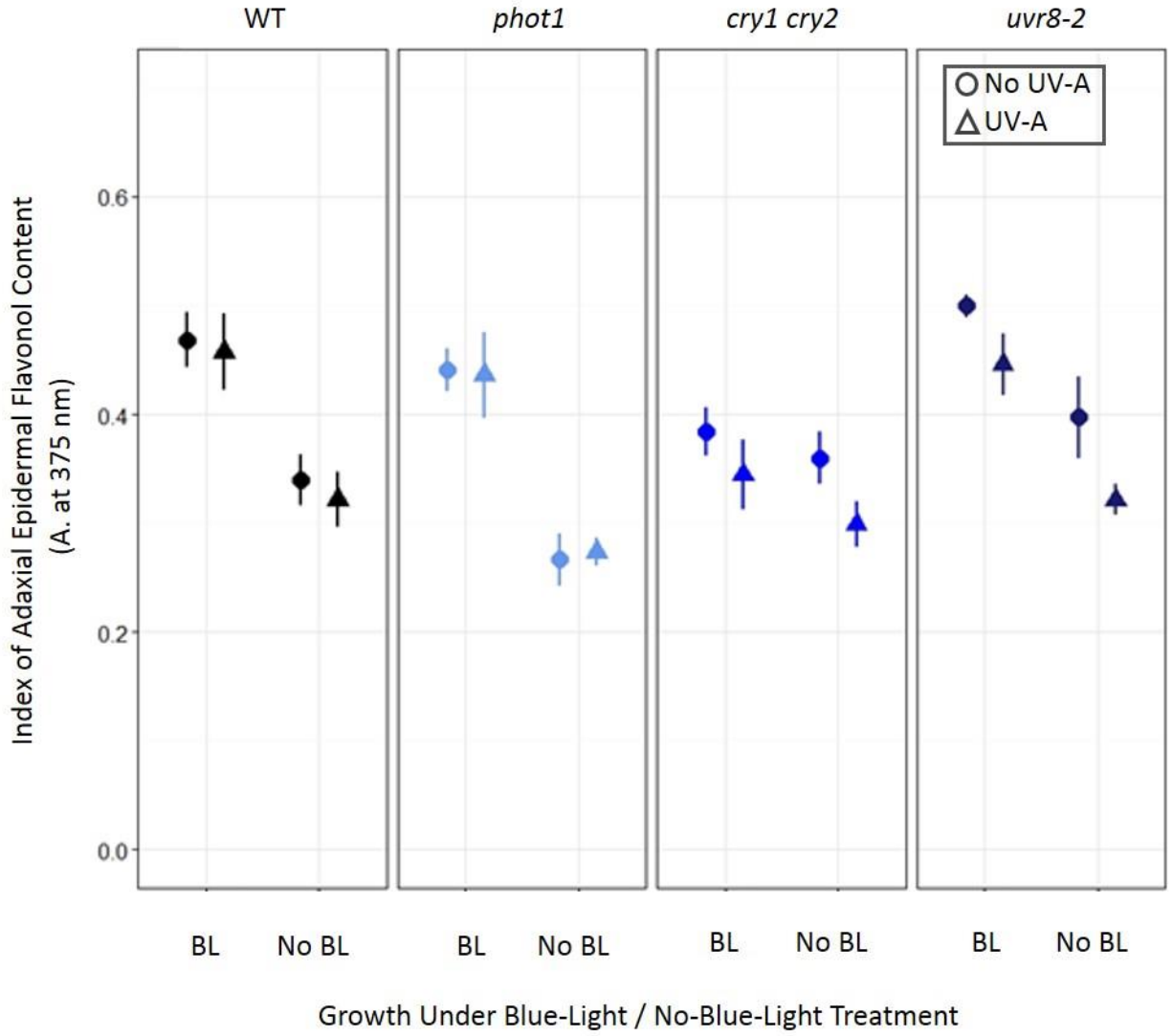
1053 Fig. 2



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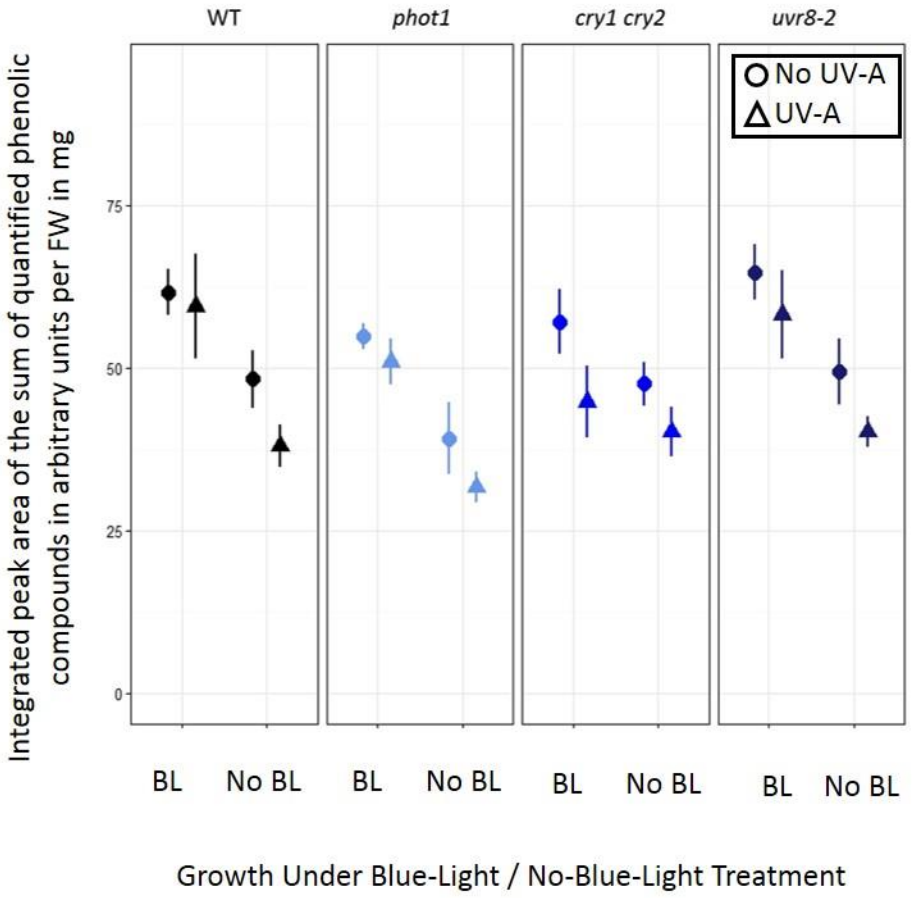
1057 Fig. 3



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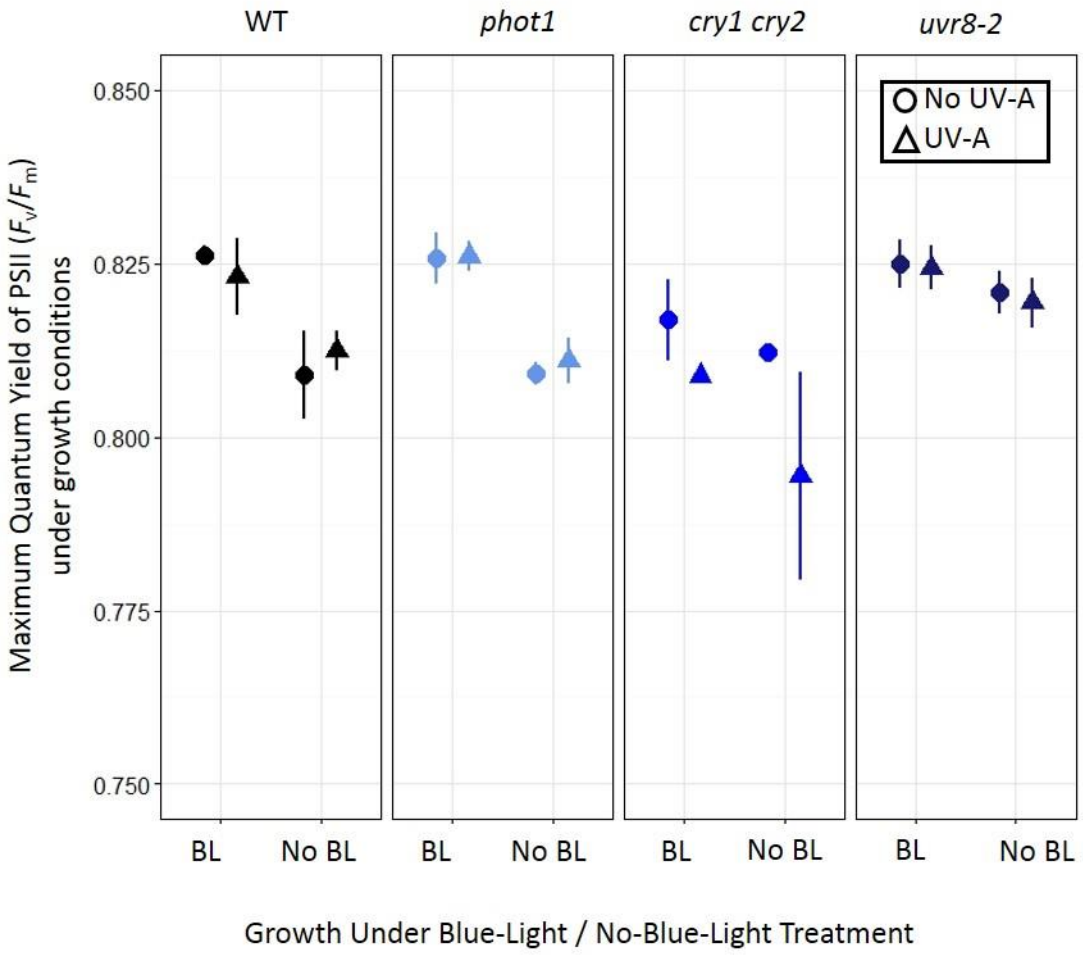
1061 Fig. 4



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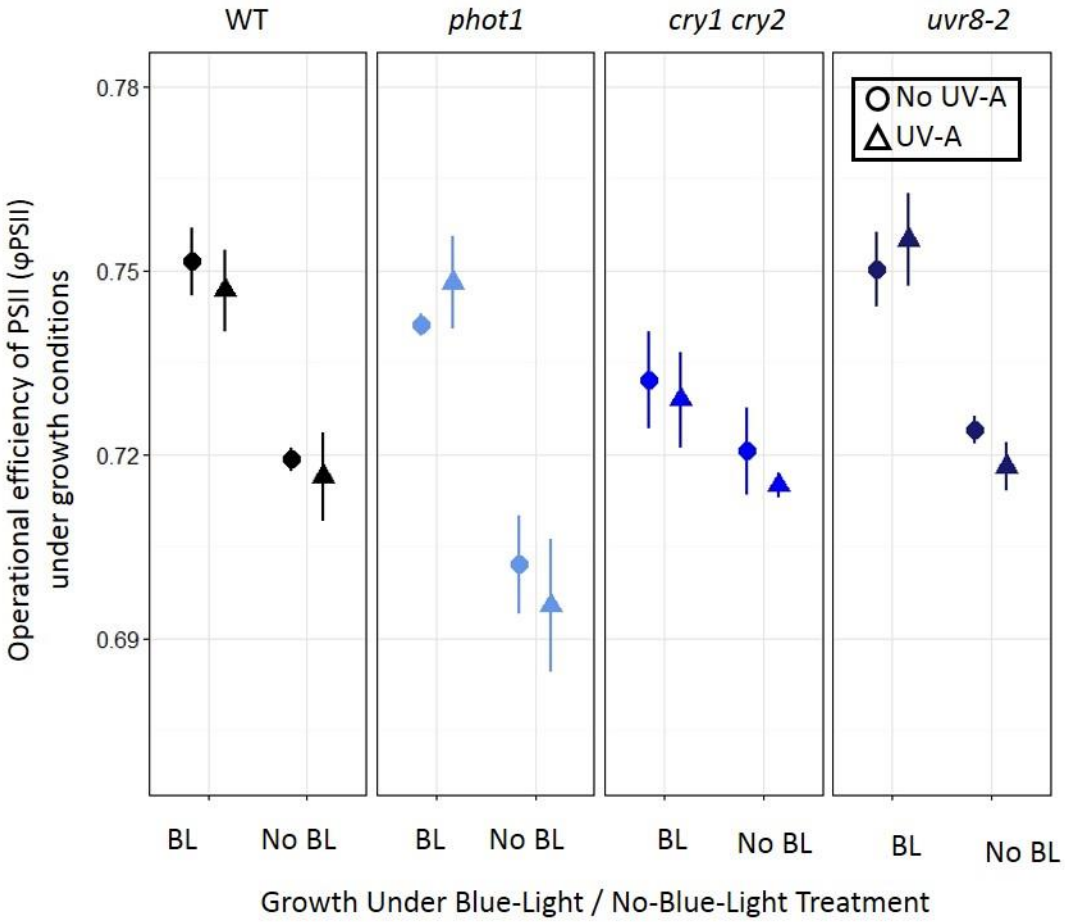
1064 Fig. 5



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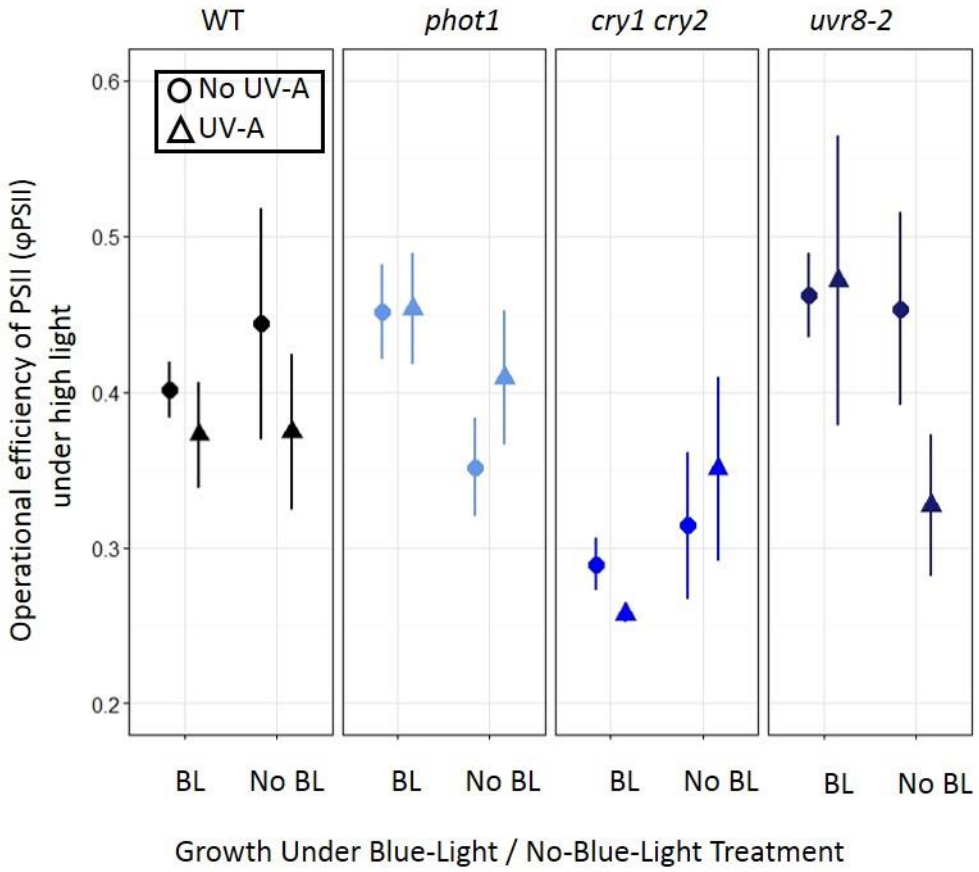
1067 Fig. 6



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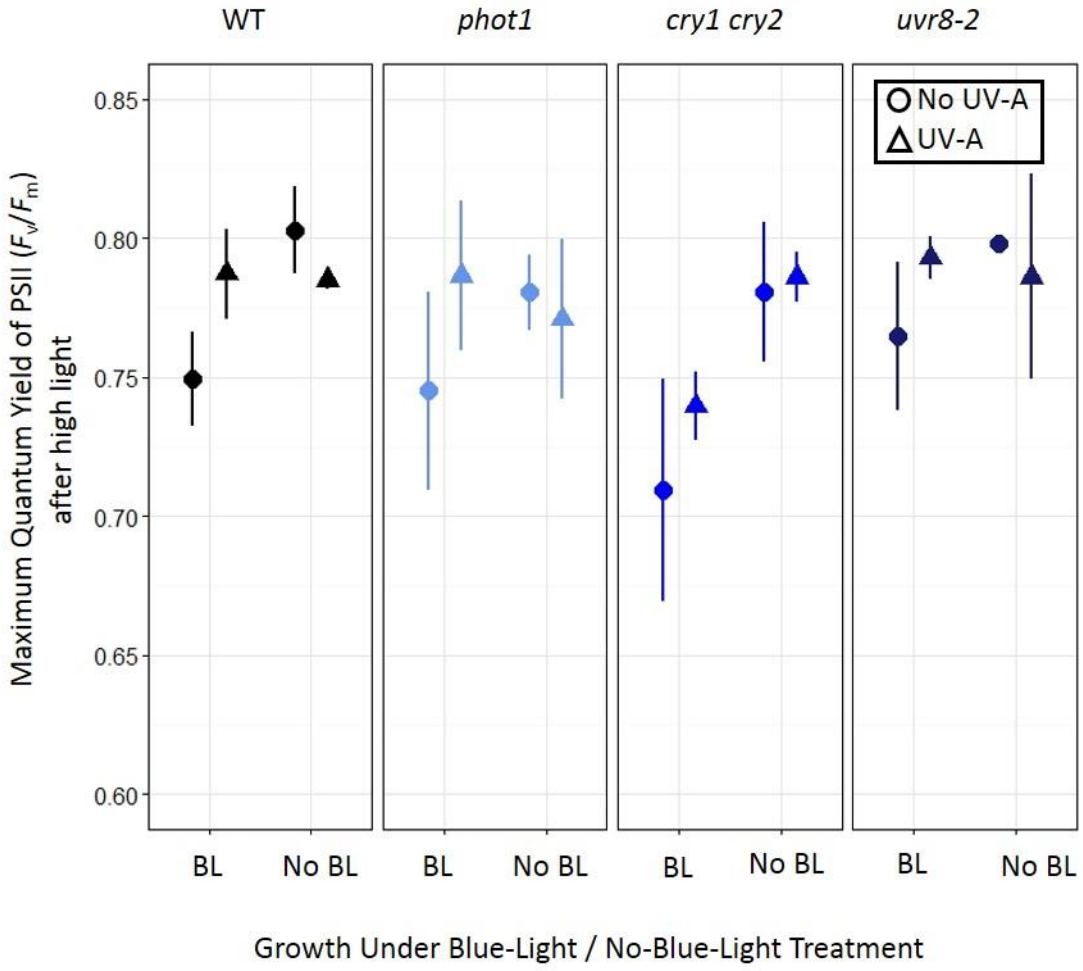
1070 Fig. 7



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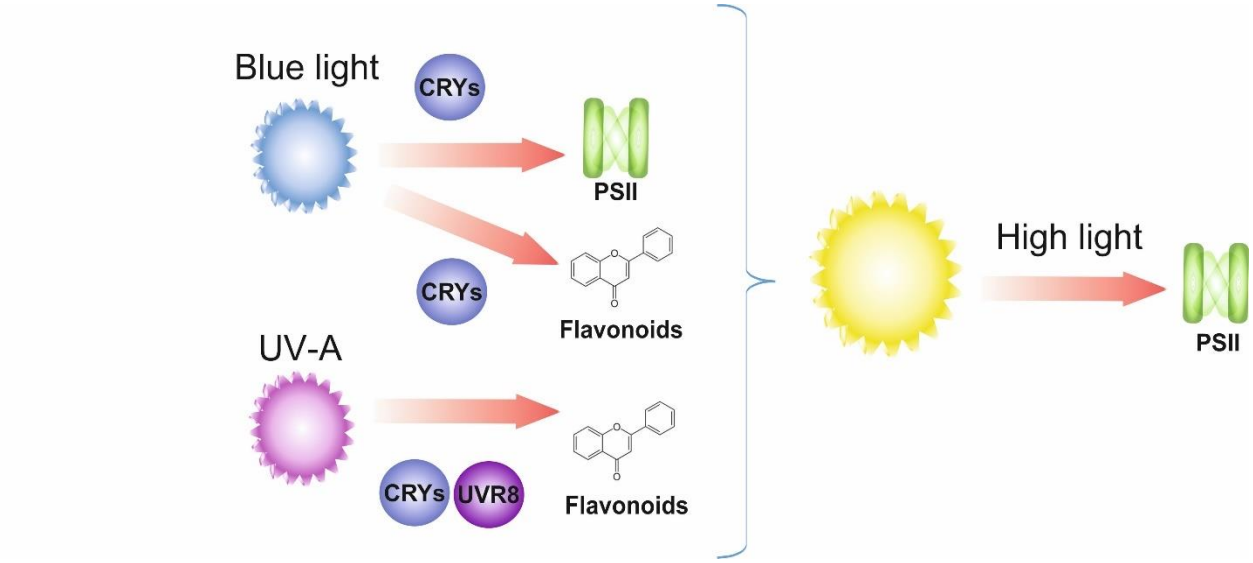
1073 Fig. 8



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